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Autophagy Pathways in CNS Myeloid Cell Immune Functions

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Abstract

The central nervous system (CNS) accommodates a diverse myeloid immune cell compartment which maintains CNS homeostasis in the steady state while contributing to tissue injury during infectious, autoimmune and neurodegenerative disease conditions. Autophagy and autophagy proteins exert fundamental roles during myeloid cell-related immune functions. Many of these tasks do not necessarily involve the canonical formation of a double-membrane structure known as the autophagosome and reflect non-canonical functions of the autophagy machinery. Here, we illustrate recent insights, concepts and outstanding questions on how autophagy pathways in myeloid cells contribute to brain health and disease.

Autophagy in the CNS: from neuronal to myeloid cells

Autophagy is a homeostatic process that enables eukaryotic cells to deliver cytoplasmic constituents for lysosomal degradation, to recycle nutrients and to survive during starvation [1]. In addition to these primordial functions, autophagy and autophagy proteins are increasingly recognized for their essential roles in the development, organization and functions of the immune system ranging from cell-autonomous defence and cell survival to coordination of complex multicellular immune responses. Many immune-related functions of conserved autophagy proteins in myeloid cells occur independently of autophagosome formation and include pattern recognition receptor-mediated uptake and phagocytosis of extracellular material for cell-surface recycling or lysosomal degradation.

Current knowledge on *in vivo* autophagy functions within the CNS largely stems from experiments targeting neuronal cells, in which absence of autophagy proteins results in the spontaneous development of early neurodegenerative disease phenotypes associated with an age-dependent accumulation of ubiquitin-positive neuronal inclusions and widespread death of neurons, in line with the classical housekeeping function of autophagy [2,3].

More recent studies demonstrated that genetic ablation of specific autophagy proteins in CNS-resident and -infiltrating myeloid cells *in vivo* can mitigate or exacerbate immune-mediated tissue injury and clinical disease development depending on the experimental condition studied. In contrast to conventional autophagy, CNS pathologies have so far predominantly been associated with non-canonical autophagy protein functions. Non-canonical pathways include LC3-associated phagocytosis (LAP) and LC3-associated endocytosis (LANDO) which require some, but not all components of the autophagy machinery and bridge receptor-mediated phagocytosis/endocytosis with autophagy. In our article, we will illustrate immune functions of autophagy proteins in myeloid cells, highlight recent data on autophagy-related functions in CNS resident and infiltrating myeloid cells during neuro-inflammatory and neurodegenerative disease conditions and discuss open questions in the field.

Autophagy proteins control myeloid cell-related immune functions

The autophagy machinery supports primarily three aspects of myeloid cell immunobiology, thereby affecting the main components of the CNS immune system. These are clearance of pathogens in a cell intrinsic manner, regulating pro-inflammatory cytokine production and antigen processing for adaptive immune system stimulation [4,5]. These functions are fulfilled by AuTophaGy (ATG) proteins [6,7] (**Figure 1**). The protein kinase complex of ULK1 (ATG1 in yeast) integrates metabolic signals and is accordingly inhibited my mammalian target of rapamycin (mTOR) and activated by the AMP activated protein kinase (AMPK). It in turn then activates the type III phosphatidylinositol 3 (PI3) kinase complex containing VPS34 and Beclin-1 (ATG6 in yeast). This kinase complex modifies membranes from which autophagosomes emerge by phosphorylating PI to PI3P. This membrane mark serves as docking site for ATG proteins that covalently link the ubiquitin-like molecules LC3A, LC3B, LC3C, GABARAP, GABARAP-L1 and GABARAP-L2 (ATG8 in yeast) to phosphatidylethanolamine (PE) in the isolation membrane that will give rise to autophagosomes. These LC3 lipidation proteins contain the E1-like enzyme ATG7, the E2-like enzymes ATG3 and 10, and the E3-like complex of ATG5, 12 and 16L1. The LC3 family of proteins facilitates membrane elongation and fusion of the double-membrane surrounded autophagosome, as well as autophagic substrate recruitment via autophagy receptors, like p62 and NDP52, that contain a LC3-interacting region (LIR) [8]. The LC3 proteins are recycled from the outer autophagosome membrane by the protease ATG4, but degraded with the inner autophagosome membrane and its cargo in lysosomes upon fusion with these hydrolytic organelles.

ATG proteins regulate cell-intrinsic, innate and adaptive immunity. Along these lines, autophagy degrades cytosolic pathogens that have escaped endosomes. This was initially demonstrated for group A streptococci [9], but also much research has focused on *Salmonella* and *Mycobacterium tuberculosis* [10,11]. These bacterial pathogens and some non-enveloped viruses escape endosomes with membrane damage. These damaged endosomes are degraded via galectin-8 mediated recognition of cytosolic exposure of their luminal glycosylation, and then degraded with their pathogen cargo by NDP52 recruited autophagy [12]. Furthermore, cytosolic bacteria are ubiquitinated and then also degraded by autophagy, often in a p62 dependent fashion. Apart from its

anti-microbial role the autophagy machinery regulates the inflammasome machinery that elicits IL-1β and IL-18 maturation and release [4]. For example, ULK1 complex dependent autophagy in the lung restricts inflammasome dependent inflammation during influenza A virus infection [13]. Moreover, absence of ATGs in myeloid cells can result in hyperinflammatory states due to the lack of damaged mitochondria clearance by autophagy resulting in reactive oxygen species (ROS) and DNA mediated inflammasome activation [14,15]. During adaptive immunity, the autophagy machinery in myeloid cells can support $CD4^+$ T cell responses by delivering substrates for antigen loading onto MHC class II molecules [16-18] and regulates $CD8^+$ and NKT cell responses through recycling of MHC class I molecules from the cell surface [19,20].

The ingenious machinery to remodel membranes and recruit effector molecules via the LC3 membrane tag is not only used for autophagosome formation during conventional autophagy, but also non-canonical functions during endo- and exocytosis, including LC3-associated phagocytosis (LAP), LC3-associated endocytosis (LANDO) and extracellular vesicle release [21,22] which can contribute to immune responses independent of autophagosome formation. In fact, many immune-related functions of conserved autophagy proteins associated with immune responses within the CNS compartment reflect non-canonical functions of the autophagy machinery as outlined below [23,24].

Myeloid cell diversity in the CNS

The CNS and its meningeal coverings accomodate a heterogeneous class of innate immune cells that contribute to the maintenance of tissue integrity and function during development and adulthood, that is only surpassed by the reactive cellular diversity during pathology [25,26]. At large, CNS-resident myeloid cell compartment can be divided into parenchymal cells such as microglia and the non-parenchymal subsets including dendritic cells and macrophage populations lining the outer barriers of the CNS, all of which occupy uniquely assigned tasks within the continuous maintenance of tissue homeostasis and immune surveillance [27,28].

Autophagy protein functions in microglia

Microglial cells are the resident immune cells of the CNS, comprising 10% of brain cells, and appear to be the only myeloid cell type within the healthy CNS parenchyma (with the exception of a few mast cells) [29]. Although they are considered CNS macrophages, studies employing single-cell transcriptomic profiling revealed that microglia are transcriptionally and epigenetically distinct from other tissue macrophages or bone marrow-derived macrophages [30] and are yolk sac-derived [31]. Microglia are long lived *in situ* and able to self-renew without significant replacement by peripheral hematopoietic cells from the blood circulation during the life of a mouse [32]. As the brain develops, microglia undergo significant transcriptional and physical changes. Early microglia express genes associated with cell proliferation, whereas early postnatal and adult microglia predominantly express genes associated with phagocytosis and immune surveillance [33]. Physiological functions of microglia include synaptic refinement by removing axons and synaptic terminals through a process termed pruning and secretion of neurotrophic factors for the support of neuronal cells [34]. Microglial cells contribute to myelinogenesis and oligodendrocyte progenitor maintenance in the early postnatal brain and mediate myelin degradation within the aging brain [35]. Thus, once thought to act simply in response to pathogens or other immune-related stimuli, microglia actively shape the circuitry of the brain and maintain CNS homeostasis.

Activation of microglial cells, a process characterized by morphological changes and upregulation of a spectrum of immune molecules such as CD11c, MHC class II, CD14, CD86, and CD44 is a hallmark of almost all degenerative and chronic immune-mediated neurological diseases and is also observed during ageing when microglia develop a proinflammatory phenotype [29,36]. Comprehensive single-cell RNA analysis of CNS immune cells in neurodegenerative conditions recently discovered disease-associated microglia (DAM), a subset of microglia showing a unique transcriptional and functional signature with downregulation of homeostatic and upregulation of genes involved in lysosomal and phagocytic pathways. Mechanisms that induce DAM and their function in CNS pathology are unclear [36]. Most studies in experimental models of CNS pathologies such as Alzheimer´s disease (AD), Parkinson´s disease (PD), and multiple sclerosis (MS) suggest that microglia activation promotes disease development and progression through dysfunctional phagocytic

activity and neuronal damage caused by the release of proinflammatory cytokines, reactive oxygen species and/or complement-activating proteins [32].

Accumulating evidence indicates that the autophagy machinery in microglia contributes to the emergence, acceleration or amelioration of CNS disease conditions. So far, two specific mechanisms appear to be predominantly relevant to CNS pathology: activation of the inflammasome **(Box 2)** leading to the consecutive release of proinflammatory mediators and autophagy protein-mediated endo-/phagocytosis.

Reduced expression of the autophagy protein Beclin-1 in microglia derived from human post-mortem AD brains suggested that lidded autophagy activity underlies impaired phagocytic receptor recycling and phagocytosis of aberrant aggregates [37]. Additionally, microglia from mice with microglial deficiency in Beclin-1 show exuberant secretion/production of IL-1β and IL-18 due to reduced autophagy-facilitated depletion of inflammasomes, thereby perpetuating degeneration-associated neuroinflammation in a transgenic mouse model for neurodegeneration that coexpresses KM670/671NL mutated amyloid precursor protein (APP) and L166P mutated presenilin 1 (PSEN1) under the control of a neuron-specific Thy1 promoter element (APPPSEN1 mice) [38]. Conversely, peroxisome proliferator activated receptor alpha (PPARA)-mediated induction of microglial autophagy has been associated with an ameliorated phenotype in the APPPSEN1 model [39]. It has been recently shown that disruption of non-canonical autophagy in microglia via targeted deletion of Rubicon and ATG5 *in vivo* leads to the increased deposition of toxic β-amyloid (Aβ) and subsequent cognitive impairment in the 5xFAD AD mouse model, while microglia-specific ablation of the essential canonical autophagy protein FIP200 had no impact. Interestingly, pharmacological inhibition of actin polymerization and phagocytosis in microglia abrogated the internalization of the prototypical LAP-substrate zymosan but left the internalization of the endocytic substrate dextran or Aβ unperturbed, supporting the notion that Aβ is predominantly taken up via the endocytic pathway rather than phagocytosis [40]. This is further supported by the fact that $LC3^{+}$ Aβ-containing vesicles colocalized with clathrin and the endosomal marker Rab5 [40].

In line with earlier work [41] the proposed underlying pathomechanism appeared to be rather a microglial deficiency in the autophagy-dependent recycling of putative Aβ

receptors (CD36, TREM2, TLR4) back to the cell surface rather than insufficient phagosome maturation and cargo destruction. However, the precise molecular underpinnings of autophagy protein-assisted receptor turnover and how the LC3 lipidation machinery is recruited to these Rab5 positive endosomes remains to be clarified. This newly characterized process, termed LC3-associated endocytosis (LANDO) which occurs through direct recruitment of the LC3 lipidation machinery to recycling endosomes and facilitates their return to the cell surface [37,40] needs to be distinguished from the previously identified non-canonical LAP initiated by recptor-mediated phagocytosis [40,42] **(Figure 2)**.

Aberrant aggregation of α-synuclein and microglial activation are closely associated with the development of PD as well as other synucleinopathies and exogenous fibrillary α-synuclein may induce autophagy in microglia [43]. A cargo-selective autophagy process in microglia designated synucleinphagy has recently been identified to promote neuroprotection by efficient clearance of neuron-derived α-synuclein in a mouse model of PD. This process depends on the TLR4-MyD88-NF-κB axis to upregulate the autophagy receptor p62, leads to the endo- and phagocytosis-independent ingestion of extracellular α-synuclein and subsequent targeting into autophagosomes therefore rendering the process to be a distinct entity from LAP and aggrephagy [44]. Microglia-specific deletion of ATG5 during development leads to a marked PD-like impairment of motor coordination in mice as young as 3 months old due to excessive inflammasome-mediated release if IL-1β and the subsequent increase of proinflammatory mediator macrophage migration inhibitory factor (MIF) [45]. In keeping with this, Han and colleagues reported restrained inflammasome activation and amelioration of neurodegeneration following small molecule-mediated induction of autophagy in mice with a PD-like phenotype [46].

A role for microglial autophagy in autoimmune CNS inflammatory diseases such as MS has not been firmely established so far. Upon activation, e.g. in response to an inflammatory milieu, microglia readily upregulate MHC class II molecules on their cell surface and autophagy could deliver substrates for MHC class II loading [16,17]. MHC class II-mediated antigen presentation by microglial cells appears, however, to be negligible for the induction of the classical T cell-driven inflammatory disease model,

experimental autoimmune encephalomyelitis (EAE) [47-49]. Complement-mediated functions such as the propagation of synaptic dysfunction, increasingly emerge as important mechanisms by which microglia participate in neuroinflammation [50]. In keeping with this, hippocampi from patients with MS depict increased expression of C1q and activated C3 in close spatial proximity to microglial protrusions [51]. Additionally, microglia are likely to calibrate CNS immune responses to various stimuli via secretion of soluble factors, which may lead to both mitigation or enhancement of inflammation. However, as to how autophagy-pathways contribute to or restrain these microgial responses in the context of both sterile and infectious neuroinflammatory disease conditions remains to be determined.

Autophagy protein functions in non-parenchymal CNS-associated myeloid cells

Bona fide dendritic cells (DCs) comprise a group of functionally and phenotypically distinct professional antigen-presenting cells that are derived from a common dendritic progenitor (CDP) and can be broadly classified into conventional DCs (cDCs) and plasmacytoid DCs (pDCs). Despite their distinct ontological segregation from other myeloid subsets, their unambiguous identification has been proven challenging due to widespread overlap of surface markers with non-DCs such as monocytes and their progeny, microglia and other macrophages, which has made it difficult to pry apart the differential contributions of these subsets especially in the context of disease [52,53]. In the steady state, CNS CD11c^{+}MHC class II^{+} DCs are sparsely distributed mainly within the leptomeninges and the choroid plexus, strictly non-parenchymal and mainly include two subsets of cDCs, while the presence and function of pDCs under this condition is subject to controversy and awaits further analysis [25,29,47]. Both, DCs depicting a surface marker expression in accordance with a cDC2 phenotype (CD11b^{+}), as well as DNGR-1/CLEC9A^{+}CD11b^{lo} DCs, resembling cross-priming cDC1s, have been identified in the choroid plexus of the uninjured brain. The latter having been also detected in the meninges [54,55]. CNS cDCs are virtually absent in the perivascular space, but readily detectable in the leptomeninges as CD11b^{+}CD172a^{+} cDC2s, whereas CD11b^{neg}CD24^{+} cDC1s dominate in the choroid plexus and can be further discerned in two subpopulations of XCR1^{+} and XCR1^{neg} cells. High-throughput scRNA-seq profiling confirmed the overall scarcity of DC subsets in the steady state CNS, their absence in the perivascular space, and the predominant presence of cDCs in the

leptomeninges and the choroid plexus but failed to detect any pDCs during homeostatic conditions in the CNS [25]. The apparent enrichment of cDCs in the leptomeninges and the choroid plexus under homeostatic conditions render these compartments as preferred entry sites for T cells to undergo DC-mediated interaction with their cognate antigen. Indeed, a role for DCs in CNS immunity is best established for T cell-mediated disease conditions.

A prototypical tool to study T cell-mediated neuroinflammation *in vivo* is experimental autoimmune encephalomyelitis (EAE), which not only serves as the pre-clinical model for MS but also permits the investigation of T cell–APC interactions in general. Before myelin-specific T cells can breach the glia limitans and infiltrate the parenchyma, they require reactivation by CNS-associated MHC class II-expressing APCs which have sampled myelin protein from oligodendrocytes [56]. Of all potential APCs isolated from the steady state CNS, only cDCs are able to present myelin antigen, which was processed *in vivo* from the unperturbed brain [57,58] and recent studies employing conditional deletion of MHC class II molecules indicated that cDCs are required for reactivating encephalitogenic $CD4^+$ T cells within the healthy CNS [25,47,58].

Genetic ablation of ATG5 in cDCs abrogates myelin peptide presentation to $CD4^+$ T cells following phagocytosis of oligodendroglial cells and completely blocks clinical EAE development induced by myelin-specific $CD4^+$ T cells [59,60]. CD11c-Cre-driven deletion of ATG7 was additionally reported to ameliorate actively induced EAE [61]. Moreover, one study using LysM-Cre mediated deletion of ATG7 in myeloid cells including DCs, found a delayed onset of EAE and accumulation of T_H17 cells in the lung during their migration to the CNS indicating that the lungs function as a central organ that licenses T cell migration into the CNS during the development of neuroinflammation [62]. However, the relative contribution of this phenotype that can be attributed to *bona fide* DCs remains to be clarified. Myelin antigen is not intrinsically expressed by DCs and, therefore, requires phagocytosis and processing in order to activate T cells. LAP does not require components of the ULK1 complex, but is often dependent of NADPH oxidase 2 (NOX2) [42,63] that is assembled at VPS34 phosphorylated membrane patches. While part of the protective effect may be attributed to non-DC myeloid cells, genetic ablation of *Cybb*/NOX2, similar to ATG5, in cDCs is sufficient to restrain encephalitogenic T cell recruitment into the CNS and to ameliorate

clinical disease development upon adoptive transfer of myelin-specific $CD4^+$ T cells [64].

These data indicate that steady state cDCs sample and process myelin antigen through LAP within the healthy CNS, supporting the reactivation of encephalitogenic $CD4^+$ T cells and subsequent EAE development. However, the Beclin 1–binding protein RUBICON has been shown to be required for LAP function [42] and global RUBICON-deficient mice show no alteration in their development of active EAE in comparison to littermate controls [65]. Therefore, DC-specific knockouts of RUBICON are warranted to further elucidate the function of LAP during CNS autoimmunity.

Non-conventional but LAP-independent immune functions of ATGs in DCs include the support of MHC class I expression and cytotoxic $CD8^+$ T cell and NKT cell responses. $CD8^+$ T cell and NKT cell responses towards infectious pathogens are compromised in the absence of ATGs [19,20,66], in part due to internalization of classical and non-classical MHC class I molecules. It remains, however, to be addressed whether these functions affect DC-mediated immune responses within the CNS.

In comparison to microglia and DCs, the ontogeny, and function of non-parenchymal brain macrophages (i.e., border-associated macrophages [BAMs]) during embryogenesis or adulthood is less well characterized [67]. There are at least three distinct non-parenchymal macrophage populations during homeostasis: leptomeningeal (mMΦ), perivascular (pvMΦ) and choroid plexus macrophages (cpMΦ) [28,68]. All three subsets of CNS endogenous macrophages are transcriptionally highly related to microglia (as opposed to peripheral monocytes and macrophages) and mostly originate prenatally from Lin^{neg}c-Kit^+ extraembryonic yolk sac-derived erythromyeloid precursors [68,69]. Tissue-resident macrophages occupy central roles during homeostasis and the differential functions of individual BAM subsets is likely to be reflected in their strategic anatomical location. While pvMΦ are involved in the development and maintenance of the CNS vasculature (including maintenance of blood-brain barrier integrity) [70,71], clear aberrant protein deposits [27,72] and, together with mMΦ help to protect the CNS from invading microorganisms [73,74],

cpMΦ are situated at the interface of the CNS and the peripheral immune system and may occupy important functions during steady state immunosurveillance, antigen-presentation and phagocytosis. Using EAE as a model for sterile neuroinflammation, a recent study documented a comprehensive transcriptional landscape across all BAM populations that elaborately reflects the dynamic signature changes in response to a specific pathological context [25]. Whether autophagy proteins regulate survival or function of non-parenchymal brain macrophages has not been adequately addressed so far, partly due to the lack of experimental tools that allow for specific targeting of BAM populations.

Concluding Remarks

Evidence that autophagy proteins in CNS myeloid cells contribute to inflammatory, vascular and degenerative brain diseases is emerging [75,76]. While current data indicate that non-canonical features of the autophagy machinery primarily execute CNS myeloid cell-related immune functions, a role for canonical autophagy pathways cannot not be exluded based on the limited data available so far. The clinical outcome of genetic ablation of autophagy proteins depends on the major immune function executed by targeted CNS myeloid cells. Autophagy deficiency in microglial cells mainly affects their ability for receptor-mediated endocytosis/phagocytosis and innate cytokine production while autophagy protein deficiency in DCs limits their efficacy to process antigen for $CD4^+$ T cell activation. Physiological functions of specialized macrophage populations at the CNS borders and how they contribute to brain pathologies are incompletely understood and the role of autophagy proteins in these cell subsets remains to be clarified. The identification of autophagy proteins as central determinants in many CNS disease models and even in human diseases represents new opportunities for therapeutic intervention. A more complete understanding how individual pathways contribute to CNS pathologies, however, appears to be mandatory in order to efficiently target autophagy pathways for therapeutic purposes (see outstanding questions).

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Text box 1: The inflammasome

Inflammasomes, as a part of the innate immune system, constitute a variety of cytosolic signaling platforms that upon sensing foreign- or host-derived stressors, initiate in a caspase-dependent manner the maturation and release of proinflammatory cytokines IL-1β and IL-18 as well as the initiation of gasdermin D-mediated pyroptosis [77-80]. These multiprotein ultrastructures regularly contain an upstream sensor molecule (such as NLRP [NLR family pyrin domain containing]1, NLRP3, NLRC [NLR family CARD domain containing]4, AIM [Absent in melanoma]2), the adaptor unit ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain or CARD) and pro-caspase 1. During canonical inflammasome activation, recognition of an adequate stimulus leads to assembly of cytosolic oligomers resulting in the proteolytic cleavage of zymogen pro-caspase 1 into its enzymatically active form [80,81], whereas non-canonical inflammasomes rely on caspase 11 (mouse)/ 4 (human) [82]. The differential stimulus specificity of individual inflammasome entities is highly dependent on the respective sensor molecule. While NLRC4 recognizes bacterial flagellin [83], NLRP1 is activated by anthrax lethal factor [84] and AIM2 by double stranded DNA [85]. Solely the NLRP3 inflammasome, to date the most fully characterized unit, has been reported to be responsive to a wide range of danger signals such as ATP, silica, alum, uric acid crystals and fibrillary proteins and peptides such as β-amyloid [79-81].

As the most abundant tissue resident innate immune cell, microglia have been the focus of interest regarding CNS-inflammasome activity and the downstream effector molecules of microglial inflammasome activation, i.e. IL-1β and IL-18, have been shown to execute pivotal functions during CNS homeostasis and pathology [86-88]. Furthermore, inflammasome-mediated promotion of pyroptosis, a proinflammatory

form of regulated cell death, is increasingly being recognized for participating in the pathogenesis of a variety of CNS conditions [89].

Autophagy pathways are implicated in the regulation of inflammasome function at various steps of their activity. At first autophagy had been identified primarily as a negative regulator of the inflammasome in that the process limits inflammasome activity by removing triggering agents, inflammasome constituents or downstream effector molecules [90-93]. However, several studies have additionally identified autophagy as an auxiliary mechanism for inflammasome activity [94,95]. Furthermore, various inflammasome constituents interact with autophagy machinery members such as Beclin-1, suggesting a reciprocal regulatory interdependency between the two platforms [96,97].

Figure legends

Figure 1: Autophagy, cell membrane dynamics. Autophagosomes emerge by isolation membrane elongation, forming for example at the endoplasmic reticulum. During autophagy induction ULK1 (1) phosphorylates VPS34 that then phosphorylates membranes for the recruitment of the LC3 or ATG8 (8) lipidation complex of Atg5, 12 and 16L1. The ubiquitin-like molecule ATG12 is activated by ATG7 and conjugated by ATG10 to ATG5. The ATG5/12/16L1 complex then ligates LC3 to the isolation membrane for substrate recruitment via autophagy receptors, like p62. LC3 or ATG8 is proteolytically processed by ATG4, activated by ATG7 and conjugated by ATG3. It is thought that LC3 influences vesicular trafficking. The majority of this lipidated LC3 or ATG8 needs however to be removed prior to fusion with lysosomes and is recycled from the outer membrane by ATG4. Syntaxin 17 (STX17) is involved in this autolysosome generation which leads to the degradation and molecular building blocks' recycling of the inner autophagosome membrane, its cargo, LC3 or ATG8 coupled to the inner membrane and any autophagy receptors attached to it.

Figure 2. LAP and LANDO as non-canonical autophagy pathways in CNS myeloif cells. (**A)** LC3-associated endocytosis (LANDO) in micoglia, modified from [40]: Clearance of fibrillary aggregates via putative Aβ surface receptors (e.g. TREM2 and CD36) followed by the consecutive lysosomal degradation of endocytosed cargo and autophagy machinery-dependent recycling of receptors back to the surface. (B) LC3-associated phagocytosis (LAP) in CNS DCs: binding of a LAP-triggering cell surface receptor (such as TIM-4, TLR2, FcγR and Dectin-1) initiates receptor-mediated phagocytosis. Activation of membrane-bound NOX2 and the consecutive production of superoxide leads to accumulation of LC3 to the cytosolic side of the LAPosome membrane. Fully assembled LAPosomes may either fuse with other endocytic vesicles or directly merge with lysosomes for subsequent MHC class II-mediated presentation to $CD4^+$ T cells.

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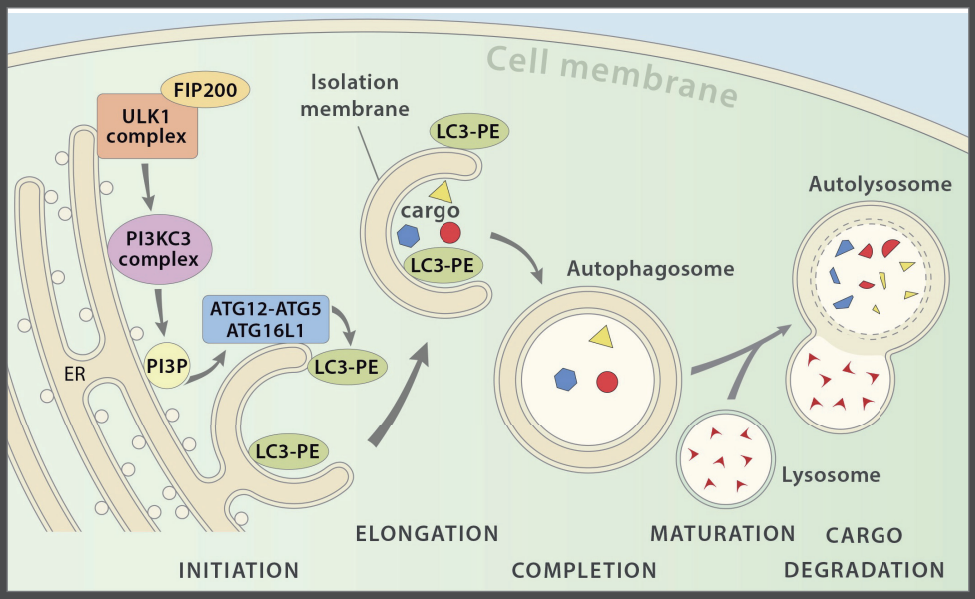
Vorlage



Anpassung

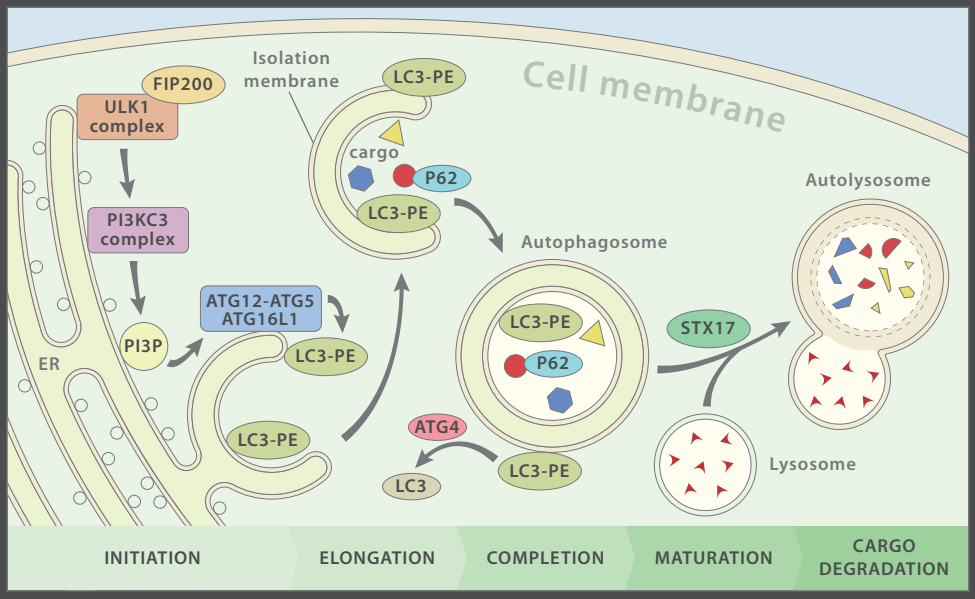


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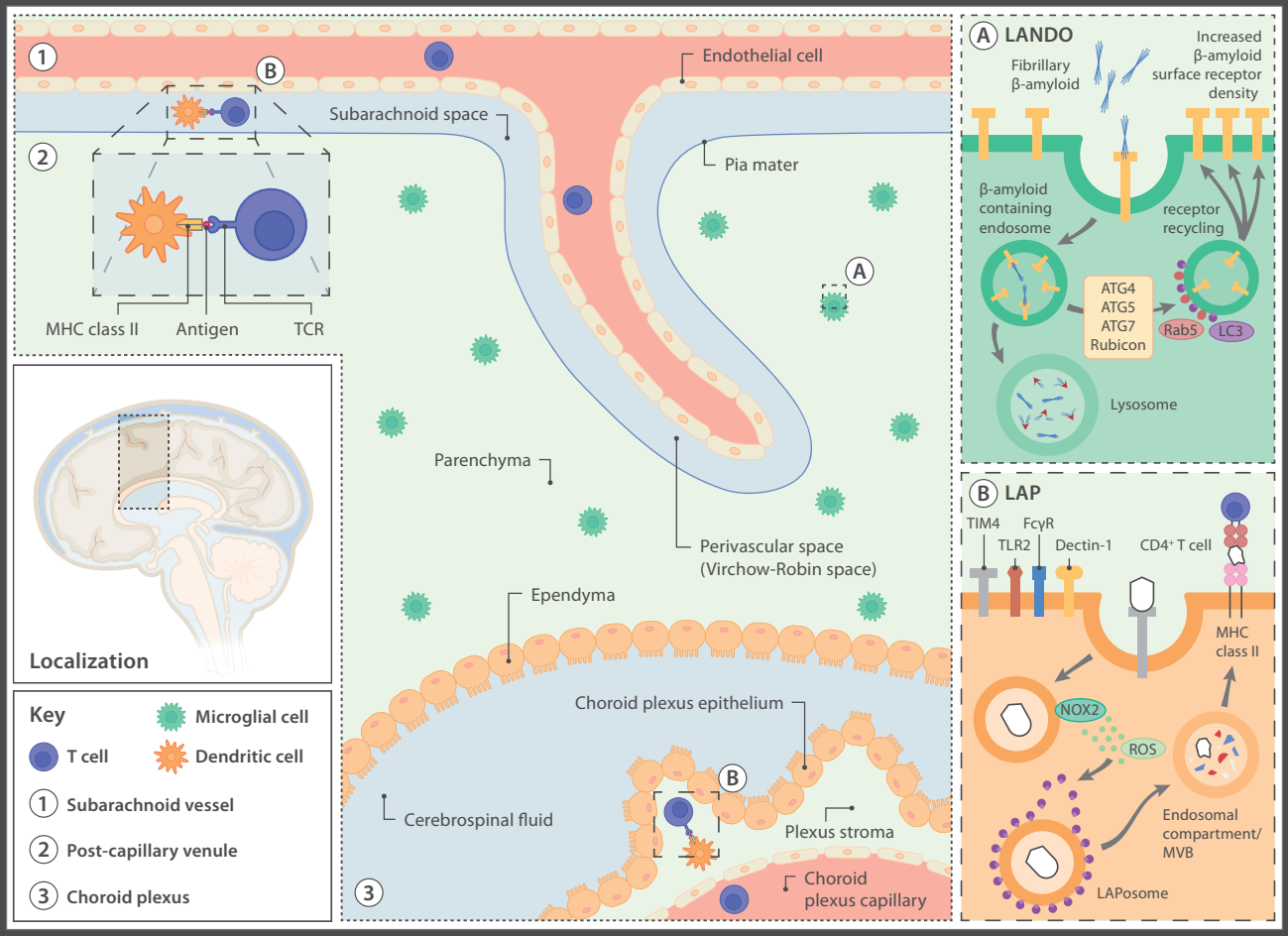
Figure 2



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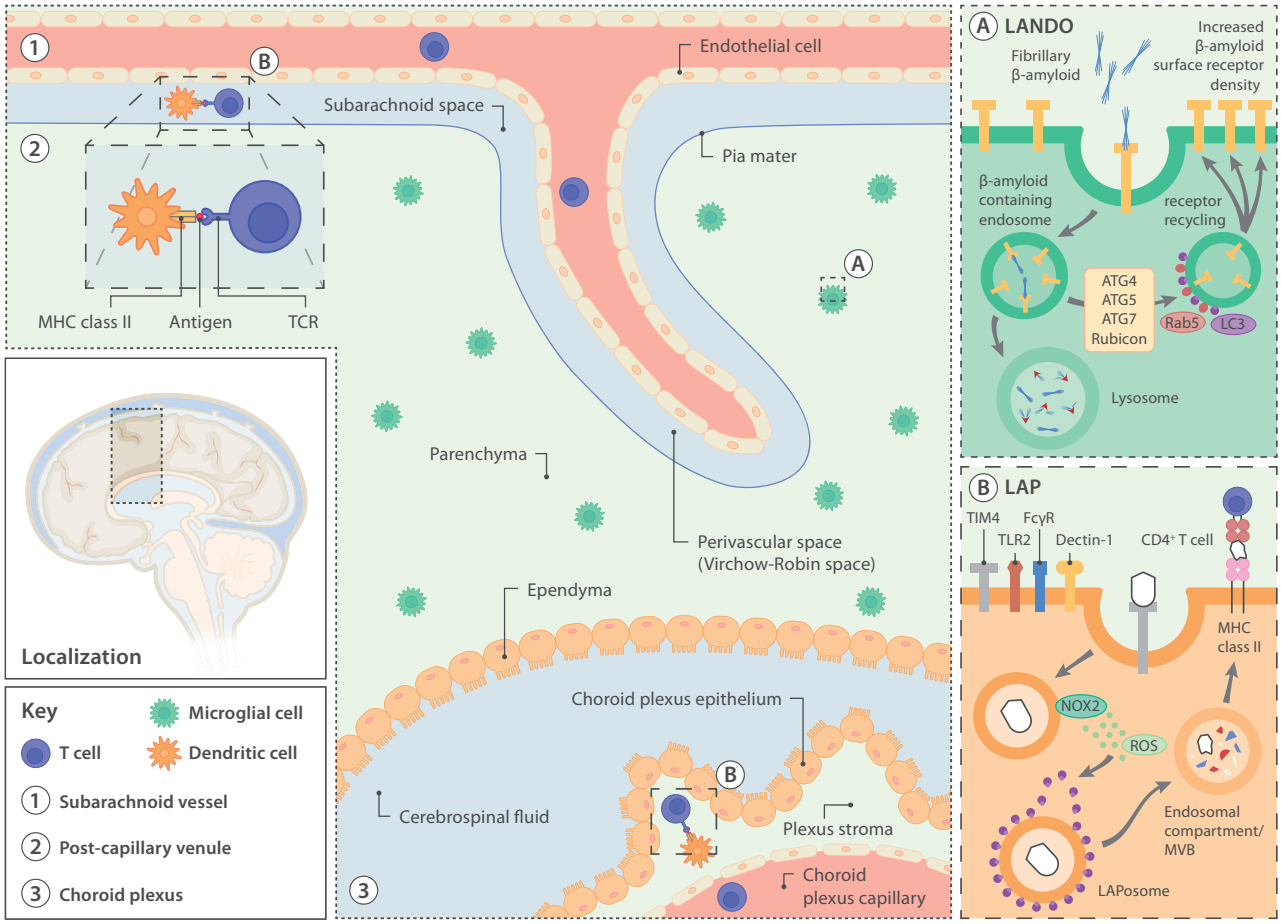

(Box 1). Which path T cells take for immune surveillance of the CNS or when they initiate an autoimmune process remain under debate.

Intravital two-photon laser scanning microscopy approaches showed that pathogenic effector T cells enter the CNS from leptomeningeal rather than CP vessels [53–55,57]. After diapedesis of the vessels, T cells encounter myelin Ag presented by meningeal APCs, which in turn triggers a local activation process ultimately leading to the formation of the autoimmune CNS lesion. Also β-synuclein-reactive T cells that induce autoimmune lesions in the cortex were recently found to use this leptomeningeal route [56]. The relevance of the leptomeningeal pathway in the initiation of EAE was also supported by earlier studies by Ransohoff and colleagues [59] and there is evidence that the meninges also represent a site of early inflammatory processes in MS [60].

However, T cells might also use alternative routes (i.e., through the CP or parenchymal vessels). It has been speculated that the molecular mechanisms for T cell extravasation are dependent on the nature of both the lymphocyte and the site of entry [52]. For example, T helper type 1 (T_H1) cells preferentially cross the BBB in the spinal cord microvessels in a VLA-4-dependent manner [61–63], while $CCR6^+$ T_H17 cells use the CP associated blood–CP barrier (BCPB) as their preferential entry site and require high-affinity leukocyte function-associated antigen (LFA)-1 for their extravasation [62,64].